

IS *CYLINDROPUNTIA* \times *FOSBERGII* (CACTACEAE) A HYBRID?

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ABSTRACT

The Mason Valley cholla, *Cylindropuntia* \times *fosbergii* (C. B. Wolf) Rebman, M.A. Baker & Pinkava, is the putative hybrid of *C. bigelovii* (Engelm.) F. M. Knuth and some other species of *Cylindropuntia*. We used AFLPs to screen chollas of the Anza-Borrego Desert in southern California to test this hypothesis of hybrid origin and identify the parental species involved. Other species scrutinized as potential parents include *C. echinocarpa* (Engelm. & J. M. Bigelow) F. M. Knuth, *C. ganderi* (C. B. Wolf) Rebman & Pinkava, *C. californica* var. *parkeri* (J. M. Coulter) Pinkava, and *C. wolfii* (L. D. Benson) M.A. Baker. Patterns of band sharing clearly testify to the close relationship between *C.* \times *fosbergii* and *C. bigelovii*. None of the other species screened came close to that level of similarity. Moreover, the numbers of total loci and unique loci in *C.* \times *fosbergii* do not meet the expectations of a hybrid taxon. We propose the alternative hypothesis that *C.* \times *fosbergii* is the sister species of *C. bigelovii*.

Key Words: AFLP, Anza-Borrego Desert, cholla, *Cylindropuntia* \times *fosbergii*, hybridization, speciation.

Long assumed to be a hybrid, *Cylindropuntia* \times *fosbergii* (C. B. Wolf) Rebman, M.A. Baker & Pinkava, the Mason Valley Cholla (also called the Pink Teddy-bear Cholla), is endemic to the Anza-Borrego Desert region of eastern San Diego County, California. It is triploid, bears fruit with aborted seeds, and exhibits substantial morphological similarities to *C. bigelovii* (Engelm.) F. M. Knuth. Although the total number of plants may only number from the hundreds to the low thousands, *C.* \times *fosbergii* can be readily spotted along California Highway S2 between Mountain Palm Springs and the ascent to Box Canyon to the west, a stretch of approximately 30 km. This cholla stands out as the tallest and pinkest cactus in the desert vegetation of Vallecito and Mason Valleys' alluvial fans.

If *C.* \times *fosbergii* is indeed a hybrid, one likely parent is *C. bigelovii* (Parfitt and Baker 1993; Rebman 1995). The two taxa are sympatric and share an erect habit featuring a single trunk with few to several main branches, and terminal segments <10 cm, 4–6 cm in diameter, and easily detached. Flower and fruit features are the same with the exception of a slight difference in inner tepal color. Both taxa are triploid, although diploid individuals of *C. bigelovii* have been found in Gila, Maricopa, and Pinal counties of south-central Arizona, and one diploid plant has

been found in southeastern Baja California (Rebman 1995; Pinkava 2002). Additionally, *C. bigelovii* and *C.* \times *fosbergii* reportedly share identical sequences for *psbA-trnH*, a spacer region of the chloroplast genome (A. Salywon, unpublished data).

Other cholla species proposed as possible parents of *C.* \times *fosbergii* are *C. echinocarpa* (Engelm. & J. M. Bigelow) F. M. Knuth (Parfitt and Baker 1993) and *C. ganderi* (C. B. Wolf) Rebman & Pinkava (Rebman 1995; Pinkava 2002). Of these two, *C. ganderi* is the most common in the habitat where *C.* \times *fosbergii* occurs, and in fact can be found growing along with *C. bigelovii* at every location where *C.* \times *fosbergii* grows. *Cylindropuntia ganderi* is shorter than either *C. bigelovii* or *C.* \times *fosbergii*, but in rare specimens it exhibits a rusty pink spine color, similar to the color of *C.* \times *fosbergii*.

Hybridization and polyploidization have been key processes in the evolution of *Cylindropuntia*, which numbers approximately 32 species (Pinkava 1999). Hybridization can result in polyploidy or serve as a step toward it (reviewed in Grant 1981). Among the North American chollas, each species is known to hybridize with at least one other species (Pinkava 2002). Further, more than 64% of the species of subfamily Opuntioideae exhibit polyploidy (Pinkava 2002). Both *C.* \times *fosbergii* and *C. bigelovii* are triploid, which could have resulted from interspecific hybridization or autopolyploidy. However, *C. bigelovii* has also been implicated as a parent, along with *C.*

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acanthocarpa (Engelm. & J. M. Bigelow) F. M. Knuth var. *major* (Engelm.) Pinkava, of the tetraploid *C. ×campii* M.A. Baker & Pinkava (Baker and Pinkava 1999). In this pairing *C. bigelovii* is proposed to have supplied an unreduced ($3n = 33$) gamete and *C. acanthocarpa* var. *major* a reduced ($n = 11$) gamete.

We undertook an investigation to determine if *Cylindropuntia* × *fosbergii* originated through hybridization between chollas of the Anza-Borrego desert. If *C. ×fosbergii* is truly of hybrid origin, then the finding of identical cpDNA sequences shared between *C. ×fosbergii* and *C. bigelovii* points to a matrilineal connection between the taxa. This assumes maternal plastid transmission in chollas, which has been found in the closely related genus *Opuntia* Mill. (Corriveau and Coleman 1988). The present work extracted data from the nuclear genome so that, if indeed *C. ×fosbergii* has a hybrid ancestry, genetic markers of both parents could be revealed. Amplified Fragment Length Polymorphisms (AFLPs; Vos et al. 1995) are ideal markers for this application, as they are biparentally inherited and rapidly evolving, and therefore can detect differentiation among even recently diverged lineages. AFLP markers contain substantial phylogenetic signal, require no knowledge about the genome under study, and, in contrast to other "fingerprinting" approaches, display high reproducibility (Koopman 2005). The use of these markers in systematic studies has been increasing, and is showing particular value in investigations of putative hybridization (Gobert et al. 2002; Segarra-Moragues et al. 2007; Errazu et al. 2009; Fjellheim et al. 2009; Yang et al. 2009). Patterns and quantities of unique and shared AFLP loci will enable assessment of the degree and nature of the relationship of *C. ×fosbergii* and other *Cylindropuntia* species of the Anza-Borrego Desert.

MATERIALS AND METHODS

Specimens

We collected multiple exemplars of *Cylindropuntia* × *fosbergii*, *C. bigelovii*, and the other candidate parental taxa, *C. echinocarpa* and *C. ganderi* (Table 1) from the Anza-Borrego Desert region. To ensure that we had considered all local candidates, however unlikely, we also included *C. californica* var. *parkeri* (J. M. Coulter) Pinkava and *C. wolfii* (L. D. Benson) M.A. Baker, species found within several miles of *C. ×fosbergii* populations. Multiple stem segments were taken from each specimen for use as voucher material (deposited at SD) and source of DNA. We extracted DNA from fresh, dried, or frozen stem material employing the protocol of Martin et al. (2006).

AFLP Analysis

We followed a modification (noted below) of the AFLP protocol of Vos et al. (1995). Each DNA sample was digested by *MseI* and *EcoRI*, then ligated with adaptors corresponding to the cuts generated by these enzymes. A first round (pre-selective) of PCR amplification used primers complementary to the adaptors but which included an additional nucleotide (*EcoRI* + A, *MseI* + C) to generate a subset of fragments. Our thermal cycler profile deviated from Vos et al. (1995) in decreasing the annealing time to 30 sec, increasing the extension time to 2 min, adding 10 more cycles, and including a final 10 min at 60°C. The second round (selective) of PCR employed primers identical to the pre-selective round but which added two more nucleotides to the adaptor sequence. A total of 18 fluorescent dye-labeled primer pair combinations generated the AFLP profiles for each sample: the *EcoRI* adaptor sequence plus AAG, ACC, and AGC; these were paired with primers with the *MseI* adaptor sequence plus CAA, CAC, CAG, CAT, CTA, and CTC. Second round thermal cycling followed Vos et al. (1995) except that we added 1 min to the extension steps. A total of 29 DNA samples were subjected to AFLP analysis: 27 exemplars and two replicates (Fos617.2 and Wolf594.2). The samples were put through the procedures in three cohorts (Cohort 1–3), partly for convenience but also as a test of the stability of experimental conditions; the replicates were assigned to different cohorts. The AFLP fragments were separated using either the ABI 310 or ABI 3100 Genetic Analyzer and a ROX fragment size standard (CSUPERB Microchemical Core Facility, San Diego State University). Chromatograms were inspected visually and peaks (loci) appearing at 30 RFU's (relative fluorescence units) or higher were scored as present or absent. All loci unique to one taxon or shared exclusively between two taxa were recorded also. To examine the patterns of AFLP variation in relation to taxonomy, morphology, and geography, the data were also subjected to principal components analysis (SPSS Statistics 17.0) and cluster analysis using neighbor-joining approach (PAUP*4.0b, Swoford 1998).

In order to deal with potential error arising from failed or spurious amplification, we created two data sets for analysis. The Total data set included all loci that were polymorphic, even if a locus was resolved in just one exemplar in one cohort. The 2/3 data set comprised loci that were resolved in at least two of the three cohorts, by at least one sample in each of the cohorts. This measure was taken because some primer combinations failed to yield any reaction in some instances.

TABLE 1. SAMPLING OF TAXA IN THE CURRENT STUDY. Exemplars are identified by abbreviated specific or varietal epithets and collection numbers; the number after the decimal point indicates the set of samples (cohort) with which it was processed. Collection site number refers to the closest mile marker along California State Route S2 to the collection location.

Taxon	Exemplar	Collection information
<i>C. ×fosbergii</i>	Fos595.3	Site 46A: Imperial Co., California Rte S-2, 0.25 mi S of intersection with Great Overland Stage Rte, 32°52'25"N 116°12'34"W, 220 m (<i>Mayer 595</i>)
	Fos599.3	Site 44: Imperial Co., California Rte S-2, intersection with Canebrake Rd, 32°54'13"N 116°13'69"W, 298 m (<i>Mayer 599</i>)
	Fos603.3	Site 46A (<i>Mayer 603</i>)
	Fos606.2	Site 42: Imperial Co., California Rte S-2, 0.45 mi N of mile marker 42, 32°56'54"N 116°17'03"W, 353 m (<i>Mayer 606</i>)
	Fos612.3	Site 28: San Diego Co., California Rte S-2, Mason Valley, 0.35 mi N of mile marker 28, 32°59'52"N 116°26'50"W, 664 m (<i>Mayer 612</i>)
<i>C. bigelovii</i>	Fos617.1/Fos617.2	Site 46A (<i>Mayer 617</i>)
	Big597.1	Site 46A (<i>Mayer 597</i>)
	Big600.2	Site 44 (<i>Mayer 600</i>)
	Big602.3	Site 46A (<i>Mayer 602</i>)
	Big605.3	Site 42 (<i>Mayer 605</i>)
	Big616.2	Site 46A (<i>Mayer 616</i>)
<i>C. ganderi</i>	Big620.3	Site 42 (<i>Mayer 620</i>)
	GandL607.3 (long spines)	Site 42 (<i>Mayer 607</i>)
	GandS610.3 (long spines)	Site 35: San Diego Co., California Rte S-2, across road from Vallecito Stage Station County Park, 32°58'35"N 116°21'01", 472 m (<i>Mayer 610</i>)
	GandS614.3 (short spines)	Site 46A (<i>Mayer 614</i>)
	GandL615.1 (long spines)	Site 46A (<i>Mayer 615</i>)
	GandS631.3 (short spines)	Site 27: San Diego Co., California Rte S-2 at crossing with Oriflamme Cyn route of the San Antonio-San Diego Mail, 33°00'27"N 116°27'23"W, 693 m (<i>Mayer 631</i>)
	GandS633.2 (short spines)	Site 46B: Imperial Co., California State Rt S2 at junction with dirt road to Indian Canyon, 32°52'46"N 116°12'40"W, 217 m (<i>Mayer 633</i>)
	GandL634.2 (long spines) GandR637.3 (red spines)	Site 46B (<i>Mayer 634</i>) San Diego Co., Carrizo Gorge Rd, 200 m N of intersection with State Hwy 94 at crossing with Carrizo Creek Rd, 32°37'24"N 116°09'31"W, 873 m (<i>Mayer 637</i>)
<i>C. echinocarpa</i>	Ech626.2	Imperial Co., California Rte S2, 2.2 km S of Anza-Borrego State Park boundary, 32°46'35"N 116°05'09"W, 262 m (<i>Mayer 626</i>)
	Ech627.1	Ibid (<i>Mayer 627</i>)
<i>C. californica</i> var. <i>parkeri</i>	Park624.1	San Diego Co., Old Hwy 80, W of Manzanita, 800 m W of intersection with Tierra Heights Rd, 32°40'29"N 116°19'34"W, 1170 m (<i>Mayer 624</i>)
	Park628.2	San Diego Co., Hwy 78, Banner Grade (<i>Mayer 628</i>)
	Park629.2	San Diego Co., California State Rte S2, in parking lot 300 m NE of intersection with Hwy 78, 33°06'06"N 116°28'28"W, 704 m (<i>Mayer 629</i>)
<i>C. wolfii</i>	Wolf594.1/Wolf594.2	San Diego Co., Mountain Springs Rd near intersection with Interstate 8, 200 m S of old highway, 32°40'26"N 116°05'56"W, 664 m (<i>Mayer 594</i>)
	Wolf635.3	San Diego Co., California State Rte S2 (<i>Mayer 635</i>)

RESULTS

The Total data set includes 692 polymorphic loci; 430 of these are present in the 2/3 data set. Loci that amplified for every specimen were not included in the data sets. The exemplars of *Cylindropuntia ganderi* exhibited the most loci in the Total data set (405); *C. ×fosbergii* displayed the fewest, but close to *C. bigelovii* (305 vs. 342,

respectively; Table 2). Perhaps due to inconsistent amplification among exemplars and cohorts, fixation within a taxon for a given locus was unusual, except for the taxa represented by just two exemplars (*C. echinocarpa* and *C. wolfii*; Table 2). Accordingly, the total number of loci amplified for a given taxon was close to the number of those loci polymorphic within a taxon (Table 2).

TABLE 2. CHROMOSOME NUMBERS OF THE SPECIES REPRESENTED IN THE PRESENT STUDY AND THE NUMBERS OF AFLP LOCI RESOLVED IN THE TOTAL DATA SET. Parenthetical numbers under Exemplars indicate a replicate. ¹Compiled by Pinkava (2002).

	Exemplars	Chromosome number ¹	Total loci	Polymorphic loci
<i>C. ×fosbergii</i>	6 (+1)	33	305	300
<i>C. bigelovii</i>	6	33 (22 uncommon)	342	341
<i>C. ganderi</i>	8	22	404	385
<i>C. echinocarpa</i>	2	22	397	249
<i>C. californica</i> var. <i>parkeri</i>	3	22	381	366
<i>C. wolfii</i>	2 (+1)	66	356	269

Exclusive sharing of loci between pairs of taxa shows virtually identical patterns between the Total data and the 2/3 data sets (Table 3); e.g., the ordering of the other taxa by degree of band sharing with *C. wolfii* was identical between the two data sets. The pairwise data underscored the close relationship of *C. ×fosbergii* to *C. bigelovii*, with which it shared more than ten times the number of exclusive loci than it shared with any other taxon (Total data, Table 3). Consequently, the analysis failed to single out any of the other species as a likely parent. Loci unique to *C. ×fosbergii* were nearly lacking in the 2/3 data set, but numbered 18 in the Total data set, a tally only surpassed by *C. bigelovii* and *C. echinocarpa* (Table 3).

The neighbor-joining tree generated with the Total data set using standard distances shows two main clusters, one consisting of *C. ×fosbergii* and *C. bigelovii*, and the other primarily bearing the remaining taxa (Fig. 1). The 2/3 data set results in a tree that is nearly identical with the Total data tree; the differences are explained in the caption of Fig. 1. Poor amplification was a problem for several specimens, primarily of Cohort 2. This lack of data depressed the similarity of these specimens to others, particularly of Cohorts 1 and 3. Consequently, the neighbor-joining tree placed several of the Cohort 2 specimens in a cluster (594W2.2, 606F.2, 616B.2, 617F2.2, 600B.2), distant from conspecific relatives (Fig. 1). This cluster included two specimens (594W2.2, 617F2.2) whose replicates were processed in different cohorts and are found in other clusters. Despite these anomalies, the tree

shows groupings of conspecific specimens from across the three cohorts of specimens, demonstrating that amplification of these markers was not typically dependent on the cohort to which a specimen belonged (Fig. 1).

The first three principal components resulting from analysis of the Total data set accounted for 25.4%, 11.9%, and 8.7% (cumulatively 46%) of the variance. Graphing the 29 samples by the first two principal components should depict intermediacy of *C. ×fosbergii* between two species that are the likely parents. The diagram (Fig. 2) depicts a close association of *C. ×fosbergii* and *C. bigelovii*, but a wide separation of these two from the other taxa. The exceptions to this pattern include two samples of *C. californica* var. *parkeri*, one *C. echinocarpa*, and one *C. wolfii* that are distributed closely around the x-axis, but distant from their conspecific relatives. These are the same samples of Cohort 2 with the anomalous placement in the cluster analysis. Again, *C. ×fosbergii* does not appear to meet the expectations of a hybrid taxon.

The collection sites for *C. ×fosbergii*, *C. bigelovii*, and *C. ganderi* along California Route S2 are noted (Fig. 1) so that the relationship between geography and genetics could be assessed. If these places are sites of current or past hybridization, we might expect clusters of exemplars of different species but from the same location. The neighbor-joining tree shows some same-species clustering by site, but no strong mixed-species clustering. One exception is the clustering of Big616.2 with Fos617.2, both of site 46A; but Fos606.2 of site 42 is very similar to

TABLE 3. TALLY OF UNIQUE AND SHARED LOCI ACROSS TAXA OF *CYLINDROPUNTIA* SAMPLES ANALYZED. The cells along the diagonal show unique loci for each taxon in the Total data set and the 2/3 data set, respectively; numbers of exclusive pairwise band sharing are shown for the 2/3 data set and the Total data set, above and below the diagonal, respectively.

	<i>C. ×fos.</i>	<i>C. big.</i>	<i>C. gan.</i>	<i>C. echino.</i>	<i>C. cal. var. park.</i>	<i>C. wolfii</i>
<i>C. ×fosbergii</i>	18/1	33	4	2	0	0
<i>C. bigelovii</i>	51	33/9	9	5	9	1
<i>C. ganderi</i>	5	10	17/7	3	10	10
<i>C. echinocarpa</i>	3	6	5	42/10	5	4
<i>C. californica</i> var. <i>parkeri</i>	0	9	24	8	18/3	5
<i>C. wolfii</i>	1	2	14	8	13	14/3

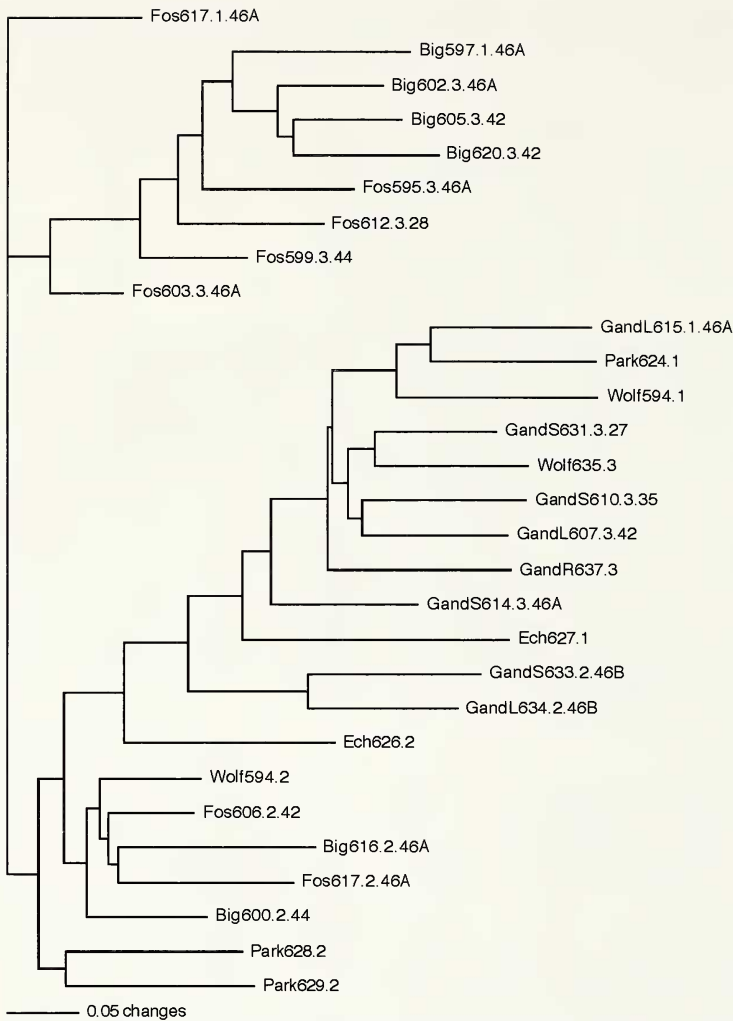


FIG. 1. Neighbor-joining tree of AFLP profiles among the *Cylindropuntia* exemplars employing the Total data set. Exemplars are identified by abbreviated epithet (Big = *C. bigelovii*, Fos = *C. ×fosbergii*, Ech = *C. echinocarpa*, Gand = *C. ganderi*, Park = *C. californica* var. *parkeri*, Wolf = *C. wolfii*), collection number, and, after the first decimal point, the group of samples (cohort) with which they were processed; the number after the second decimal point denotes the collection site (Table 1). Exemplars Fos617.2.46A and Wolf594.2 are replicates of Fos617.1.46A and Wolf594.1, respectively. The only topological differences between this tree and the tree resulting from the 2/3 data analysis are (1) the branch bearing GandS633.2 and GandL634.2 is found in the same phyletic grade but between the branches bearing GandR637.3 and GandS614.3, and (2) the positions of Fos606.2 and Fos617.2 are switched.

these two and switches positions with Fos617.2 in the topology of the 2/3 data tree (Fig. 1).

DISCUSSION

The results clearly depict *Cylindropuntia ×fosbergii* and *C. bigelovii* as each other's closest relative. The current prevailing view is that *C. ×fosbergii* is the hybrid derivative of *C. bigelovii* and some other species. If this hypothesis is to be supported on the molecular level, one should expect a substantial amount of genetic material shared exclusively between parent and hybrid. The high numbers of loci limited to specimens of

C. ×fosbergii and *C. bigelovii* indeed meet this expectation (Table 3). However, no other species has emerged as a likely second parent; patterns of band sharing suggest a relatively distant relationship of *C. ×fosbergii* with either *C. ganderi*, *C. echinocarpa*, *C. californica* var. *parkeri*, or *C. wolfii*. A stronger similarity of *C. ×fosbergii* to *C. bigelovii* might be expected if, as has been suggested (Parfitt and Baker 1993), the triploid *C. ×fosbergii* received two sets of chromosomes from *C. bigelovii*. But even if a highly heterozygous *C. bigelovii* was a parent of *C. ×fosbergii*, that still could not account for the 10-fold difference in band sharing observed between *C.*

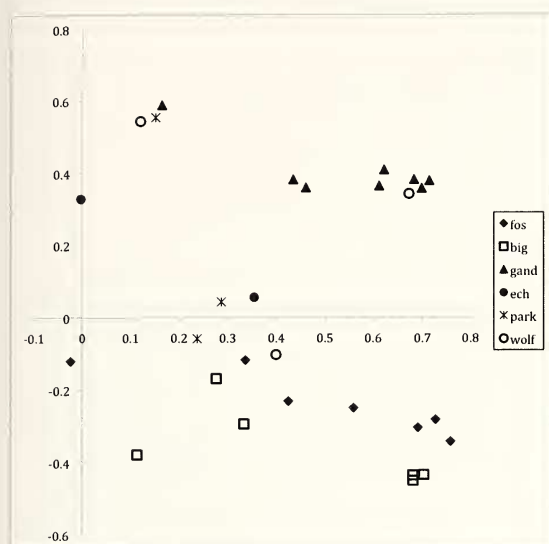


FIG. 2. Scatter diagram of the exemplars by principal components 1 (x-axis) and 2 (y-axis). Taxon abbreviations follow Fig. 1.

\times *fosbergii* and *C. bigelovii* versus any other pairing with *C. \times fosbergii* (Table 3).

Hybrids, relative to their parents, are expected to possess few or no loci not found in one of their parents, unless the hybridization event was ancient enough for unique mutations to accumulate in the hybrid taxon. Moreover, a sterile triploid status, as in *C. \times fosbergii*, virtually rules out gaining genetic variation via gene flow or recombination. *Cylindropuntia \times fosbergii* possesses unique alleles, albeit fewer than *C. bigelovii* but comparable in number to *C. californica* var. *parkeri*, *C. wolfii*, and *C. ganderi* (Total data, Table 3). The discrepancy between the Total data and the 2/3 data sets regarding the numbers of unique loci in *C. \times fosbergii* most likely reflects a highly restricted distribution of unique loci in *C. \times fosbergii*; i.e., because most of its unique alleles are limited to a single specimen, most of these loci were not included in the 2/3 data set. If *C. \times fosbergii* is indeed a hybrid, it is possible we have overlooked another, possibly extinct, taxon that transmitted its unique loci to *C. \times fosbergii*.

Another hallmark of hybrids, especially F_1 , is a high level of heterozygosity or numbers of loci, or both. Because AFLPs are dominant markers, a hybrid would be expected to exhibit more total bands (alleles/loci) compared to representatives of the parent species. Of all the taxa surveyed in this study, *C. \times fosbergii* exhibits the fewest number of loci, counter to the expectation of hybrid status (Table 2).

The sterile, triploid status of both *C. \times fosbergii* and the Anza-Borrego populations of *C. bigelovii* makes contemporary formation of hybrids or hybrid swarms involving these taxa unlikely.

Moreover, the unique loci and genetic divergence of *C. \times fosbergii* specimens from a single location seem to rule out anything but an ancient hybrid origin of the taxon. If a unique hybridization event produced the triploid *C. \times fosbergii*, most subsequent reproduction of *C. \times fosbergii* would be expected to be vegetative (by detached stem segments). In this case, genetic variation among individuals is expected to be low or nil. If, however, hybridization leading to a triploid *C. \times fosbergii* was recurrent and contemporary, then variation among the hybrids could be higher, but patterns of similarity would be expected to show congruency between the distribution of the parents and the hybrids. Patterns of relationship within and between *C. \times fosbergii* and *C. bigelovii* or *C. ganderi* do not show strong geographic structuring along the stretch of California Route S2 where the three co-occur (Fig. 1), which is expected if the collection sites are present or past hybrid zones. Moreover, there are no known diploid *C. bigelovii* in California, and the triploid forms have not been shown to produce sexual offspring, with the exception of the formation of *C. \times campii*, which arose from the union of an unreduced triploid gamete of *C. bigelovii* and a reduced haploid gamete of *C. acanthocarpa* var. *major* (Baker and Pinkava 1999).

Alternatively, the findings of the present study can be construed to support the hypothesis of a sister-species relationship between *C. \times fosbergii* and *C. bigelovii*. Both species are triploid ($3n = 33$) and propagate vegetatively, except that *C. bigelovii* fruit has been observed to occasionally produce a seed (Rebman 1995), but it is unclear if these are produced sexually or asexually. The numbers of polymorphic loci and unique loci in *C. \times fosbergii* and the other taxa in the study are comparable. A peripatric process may have led to the origin of *C. \times fosbergii* from ancestral *C. bigelovii*. *Cylindropuntia bigelovii* is a common cholla of the Sonoran Desert of Arizona, California, Sonora, Baja California, and Baja California Sur, whereas *C. \times fosbergii* is a narrow endemic, existing in a few small groups in one valley system on the western edge of *C. bigelovii*'s range. The widespread *C. bigelovii* exhibits more unique loci, greater polymorphism, and at least a two-fold greater similarity to the other taxa in the study. *Cylindropuntia \times fosbergii* may have evolved from isolated populations of *C. bigelovii* and eventually suffered range contraction, bottlenecks, and the resulting decline in genetic variability. The Anza-Borrego region has been a desert for only about 15,000 years (reviewed in Lindsay and Lindsay 1991), and whether or not this provides the time for these processes to occur is unclear.

The data presented do not support the notion that the AFLP patterns are simply a function of sample size or ploidy of the taxa. For example,

diploid *C. ganderi* exhibited the largest number of polymorphic loci but was represented by the largest number of exemplars. The larger sampling ($n = 8$) resulted from our desire to assess the significance of spine length and color variation in *C. ganderi*. Despite this larger sampling, *C. ganderi* displayed one of the lowest numbers of unique alleles and did not cluster near *C. ×fosbergii*. Plants from the same location collected with short or long spines clustered together (Fig. 1: Gand633.2 + Gand634.2), suggesting only a minor genetic or developmental component underlying this difference. Further, *C. ganderi* plants with the rare red spine color (e.g., GandR637.3) do not seem to be the direct source for the similar spine color in *C. ×fosbergii*, and also does not appear to represent a lineage independent of *C. ganderi* (Fig. 1). *Cylindropuntia wolfii* exhibited the lowest number of polymorphic loci but is a hexaploid, whereas *C. echinocarpa* is a diploid, represented by just two individuals, and displayed the most unique loci (Tables 2, 3). However, *C. wolfii* has a relatively small distribution on the western edge of the Sonoran Desert and produces generally sterile seeds whereas *C. echinocarpa* is sexual species with a range that extends from California and Baja California to Nevada, southwestern Utah, Arizona, and Sonora.

It is clear that AFLP data has great potential for phylogenetic questions in groups with low levels of divergence (Koopman 2005). Moreover, it has been used convincingly to support (Segarra-Moragues et al. 2007) or refute (Yang et al. 2009) hypotheses of hybridization. In the present study, we find that the large reservoir of potential AFLP data can help compensate for the problem of missing data, as evidenced by the similar results from analyses of the 2/3 vs. the Total data sets. These data have successfully excluded all but *C. bigelovii* as a possible parent of *C. ×fosbergii*, but provide no additional support for the hybrid origin hypothesis; instead, the patterns are more suggestive of a sister status of these two taxa.

Cylindropuntia ×fosbergii should be recognized as a species rather than a hybrid. Its unique stature and spine color in the Anza-Borrego Desert do not appear to be the genetic legacy of any extant species. Thus, even if *C. ×fosbergii* is the product of an ancient hybridization event, it harbors a unique combination of genetic information, leaving it distinct among cholla species.

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